Diagnostic criteria for monoclonal B-cell lymphocytosis

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Summary
Very low levels of circulating monoclonal B-cell subpopulations can now be detected in apparently healthy individuals using flow cytometry. We propose the term ‘monoclonal B-cell lymphocytosis’ (MBL) to describe this finding. The aim of this document is to provide a working definition of MBL for future clinical, epidemiological and laboratory studies. We propose that the detection of a monoclonal B-cell population by light chain restriction is sufficient to define this condition in individuals not meeting the diagnostic criteria for other B-lymphoproliferative disorders. The majority of individuals with MBL will have cells that are indistinguishable from chronic lymphocytic leukaemia (CLL). However, this blood cell clonal expansion of CD5+ or CD5+ B-lymphocytes is age-dependent and immunophenotypic heterogeneity is common. Longitudinal studies are required to determine whether MBL is a precursor state to CLL or other B-lymphoproliferative disease in a situation analogous to a monoclonal gammopathy of undetermined significance and myeloma. Future studies of MBL should be directed towards determining its relationship to clinical disease, particularly in individuals from families with a genetic predisposition to developing CLL.

Keywords: monoclonal B-cell lymphocytosis, B cells, early detection, surrogate biomarker, familial chronic lymphocytic leukaemia.

The increasing technological ability to detect monoclonal B cells using three- and four-colour flow cytometry has led to the identification of very low levels (<0.005 x 10⁹ cells/l) of circulating clones of B cells with surface features similar to chronic lymphocytic leukaemia (CLL) in apparently healthy individuals. Such clones are now being detected within the context of an absolute lymphocyte count of 5.0 x 10⁹/l or below the level required for a diagnosis of CLL. If these clonal expansions are implicated in the aetiology of CLL then their study has the potential to offer insight into the molecular development of CLL in general. A variety of terms have entered the literature to designate this finding. Followed by a meeting of the International Workshop on CLL at the National Cancer Institute (NCI) in Bethesda, MD, a subcommittee of the International Familial CLL consortium was formed to summarize the literature and to propose a unified nomenclature to describe the finding of a monoclonal expansion in healthy individuals. Here, we define monoclonal B-cell lymphocytosis (MBL) as the flow cytometric detection of a light chain restricted lymphocytosis. In addition to providing a working definition of MBL we suggest areas for further study.

History and population prevalence of MBL
Chronic lymphocytic leukaemia is defined by the presence of monoclonal B-lymphocytes co-expressing CD19, CD5 and CD23, with weak or no expression of CD20, CD79b, FMC7 and surface immunoglobulin. The monoclonal B cells must represent the majority of leukocytes with an absolute lymphocyte count >5 x 10⁹ cells/l, which has persisted for at least 1 month (Cheson et al, 1996). Several investigators have demonstrated that patients fitting within these criteria had disease that was stable for long periods of time, and have used a variety of different names to denote this situation. It has also...
been noted that patients with CD5⁻ B-cell disorders may have stable disease. These reports are characterized in Table I. These studies have been critical to the understanding that there is a spectrum of disease in CLL and other B-cell lymphoproliferative disorders, both in terms of the tumour burden at presentation and the probability that the disease will progress to a stage that requires treatment. The application of basic diagnostic flow cytometry to the general population has led to the detection of a MBL in otherwise healthy individuals.

Monoclonal B cells in healthy individuals with normal peripheral blood counts were first noted in studies of unaffected siblings families with a genetic predisposition to CLL (Marti et al, 1992a,b; Marti, 1993). In 1995, a US Public Health Service (USPHS) Workshop reviewed the laboratory, clinical, and population data and recognized that both CD5⁻ and CD5⁺ MBL could be identified in healthy individuals and outpatients with no clinically apparent evidence of haematological disease (Cartwright, 1997; Marti et al, 1997; Sarasua et al, 1997; Vogt et al, 1997). In a large cross-sectional population study of 1491 individuals (Marti et al, 1997; Sarasua et al, 1997; Vogt et al, 1997), US investigators at the Agency for Toxic Substances and Disease Registry, Centres for Disease Control and the Food and Drug Administration found evidence of a MBL phenotype in 11 individuals (0.8%). Research from the Haematological Malignancy Diagnostic Service Laboratory using three-colour flow cytometry demonstrated a higher prevalence of approximately 1.7% in an outpatient clinic population (Jack et al, 1997). Following recommendations from the USPHS Workshop, Slade (1999) selected patients with an absolute B-cell lymphocytosis or relative increase in the proportion of CD5⁺ B cells for monoclonality assessment, and demonstrated a prevalence of 0.6% (13/1895). More recently, Rachel et al (2002) examined MBL using a similar screening strategy in a study of mid-Western US blood donor population. Among donors aged 39–80 years, they found a prevalence of MBL of 0.14% (7/5138). These studies demonstrated that an MBL may be detected in the absence of an absolute lymphocytosis and in individuals with a normal proportion of CD5⁺ B cells. However, the reported prevalence varied dependent on the technique used, and the development of CLL-specific assays suitable for minimal residual disease detection that are more sensitive than polymerase chain reaction for immunoglobulin heavy chain rearrangement (IgH-PCR) (Rawstron et al, 2001) potentially allows the detection of monoclonal B cells in individuals with an apparently normal kappa:lambda ratio.

Using this assay, Rawstron et al (2002a) detected CLL-phenotype cells in 2.7% of adults aged over 18 years (0.3% in individuals younger than 40 and 5.2% in individuals older than 60). Incidental to CLL-specific assay, these studies identified CD5⁻ non-CLL phenotype MBL in nine of 910 (1.0%) individuals over 40 (Rawstron et al, 2002a). Using a similar approach in a population-based study of 500 normal Italian subjects over the age of 65 years with a normal blood cell count, Ghia et al (2004) found 29 individuals with a clonal expansion of lymphocytes with either a CLL-phenotype (n = 22/442, prevalence 5.5%) or CD5⁻ non-CLL-phenotype (n = 7/500, 1.4%) showing good consensus between the two independent studies. All cases, regardless of the phenotype, were CD10 negative and BCL1 or BCL2 rearrangements were not found. In both studies, a monoclonal IgH rearrangement was confirmed by IgH-PCR in the majority of cases. The absolute monoclonal B-cell count ranged from 0.002–1.5 × 10⁹/l. There was a male predominance, and an increasing prevalence with age similar to that observed in clinical disease. It has also been demonstrated that the CLL-phenotype cells in otherwise haematologically normal outpatients have similar karyotypic abnormalities to clinical disease with respect to 13q14 deletions (O’Connor et al, 2003).

In summary, studies assessing light chain clonality only will identify MBL in approximately 0.5–1% of the adult population. Studies combining a disease-specific phenotype with clonality assessment detect MBL in approximately 2–3% of the population. The prevalence increases with age, rising to over 5% for adults aged 60 years or older.

**Diagnostic criteria**

The diagnosis of MBL is based on the identification of a clonal lymphocyte population by immunophenotypic characterization. Different laboratories have used diverse approaches to identify minimal B-cell monoclonal lymphocytosis, making comparisons across geographic, ethnic, and in different risk groups difficult. In order to standardize and facilitate future studies, we propose the following set of guidelines for the diagnostic characterization of a blood B-cell monoclonal lymphocytosis.

1. Detection of a monoclonal B-cell population in the peripheral blood with
   i. overall kappa:lambda ratio >3:1 or <0:3:1, or
   ii. greater than 25% of B cells lacking or expressing low level surface immunoglobulin or
   iii. a disease-specific immunophenotype.

2. Repeat assessment should demonstrate that the monoclonal B-cell population is stable over a 3-month period.

3. Exclusion criteria
   i. lymphadenopathy and organomegaly, or
   ii. associated autoimmune/infectious disease, or
   iii. B-lymphocyte count >5 × 10⁹/l, or
   iv. any other feature diagnostic of a B-lymphoproliferative disorder. However, a paraprotein may be present or associated with MBL and should be evaluated independently.

4. Subclassification:
   i. CD5⁻23⁻: this is the major subcategory and corresponds to a CLL immunophenotype (Cheson et al, 1996).
   ii. CD5⁻23⁺: correlate moderate level of CD20 and CD79b expression with atypical CLL.
   iii. CD5⁺: corresponds to non-CLL lymphoproliferative disease (LPD).
<table>
<thead>
<tr>
<th>Term</th>
<th>Subjects and study setting</th>
<th>Definition</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Benign monoclonal B-cell lymphocytosis</td>
<td>20 of 500 CLL cases</td>
<td>Rai stage 0, no progression</td>
<td>Han et al (1984)</td>
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<tr>
<td>Benign monoclonal B-lymphocytosis</td>
<td>327 of 1777 CLL patients</td>
<td>Rai stage 0, no deep nodes</td>
<td>Mandelli et al (1987)</td>
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<td>B-monoclonal lymphocytosis of undetermined significance (BMLUS)</td>
<td>25 patients in one study</td>
<td>Blood and bone marrow only decreased</td>
<td>Aguilar-Santelises et al (1989, 1992); Garcia et al (1989); Kimby et al (1989); Aman and Mellstedt (1991)</td>
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<td>Smoldering CLL</td>
<td>127 of 261 CLL did not progress; 31% progression at 3 years</td>
<td>Hb ≤13 g/dl, ALC &lt;30 × 10^9/l and LDT &gt;12 months</td>
<td>Montserrat et al, 1988</td>
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<td>Smoldering CLL (French Co-operative Group)</td>
<td>231 of 309 natural history Binet stage A CLL; two groups defined from two studies</td>
<td>A1*: Hb &gt;12 g/dl, ALC &lt;30 × 10^9/l; A1**: Hb &lt;12, ALC &gt;30 × 10^9/l</td>
<td>French Cooperative Group on Chronic Lymphocytic Leukemia (1990)</td>
</tr>
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<td>B-cell monoclonal lymphocytosis</td>
<td>Two CLL kindreds</td>
<td>Downregulation of CD20</td>
<td>Marti et al (1992a); Marti (1993)</td>
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<td>B-cell monoclonal lymphocytosis</td>
<td>Residents in areas near hazardous waste dumps; stratified random sample by age and sex; 10 USA sites, 1991–4; age &gt;45 years; 11 of 1499 (0.7%); age &gt;40 years; estimated 1.5–2% of Yorkshire hospital patients</td>
<td>B-cell-like phenotype defined as lymphocytosis with B-lymphocytes &gt;50 percentile, CD20 dim, abnormal CD5</td>
<td>Vogt et al (1997)</td>
</tr>
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<td>B-cell monoclonal lymphocytosis</td>
<td>Same base population as Vogt et al (1997); three of 11 subjects with increased B cells</td>
<td>Monoclonality demonstrated by kappa-lambda analysis</td>
<td>Marti et al (1997)</td>
</tr>
<tr>
<td>B-cell monoclonal lymphocytosis</td>
<td>Clonal B-population in subjects age &gt;40 years estimated 1.5–2%; 1000 Yorkshire hospital outpatients</td>
<td>Three-colour flow cytometry with light chain expression</td>
<td>Jack et al (1997)</td>
</tr>
<tr>
<td>Detectable, sub-clinical BCLL B-cell monoclonal lymphocytosis</td>
<td>37 Samples showed light chain restriction with normal morphology</td>
<td>87% Confirmed by PCR analysis for Ig gene rearrangement</td>
<td>Maiese and Braylan (1997)</td>
</tr>
<tr>
<td>B-cell monoclonal lymphocytosis</td>
<td>13 of 1985 (0.6%)</td>
<td>B-cell follow-up study</td>
<td>Slade (1999)</td>
</tr>
<tr>
<td>CLL-like immunophenotype non-CLL-like immunophenotype</td>
<td>32 of 910 (3.5%) clinic outpatients</td>
<td>Four-colour flow cytometry Ig gene spectral typing</td>
<td>Rawstron et al (2002a)</td>
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<td>CLL-like immunophenotype non-CLL-like immunophenotype</td>
<td>Eight of 59 (13.5%); apparent unaffected cases from 21 CLL kindreds</td>
<td>Four-colour flow cytometry</td>
<td>Rawstron et al (2002b)</td>
</tr>
<tr>
<td>B-cell monoclonal lymphocytosis</td>
<td>Seven of 5138 (0.14%); adult blood donors</td>
<td>Three-colour flow cytometry; PCR IgH rearrangement</td>
<td>Rachel et al (2002)</td>
</tr>
<tr>
<td>CD5 negative BMLUS</td>
<td>Seven patients stable chronic lymphocytosis</td>
<td>CD5&lt;sup&gt;-&lt;/sup&gt;, CD23&lt;sup&gt;-&lt;/sup&gt; not viewed as precursor CLL</td>
<td>Wang et al (2002a, b)</td>
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More than one set of kappa/lambda light chain reagents may be used; the detection of any B-cell monoclonal population by light chain restriction is sufficient. Confirmation with IgH-PCR may be helpful but is not essential.

2 The monoclonal B-cell population may represent a minority of total B cells when identified by a disease-specific immunophenotype. These may be demonstrable even if the overall kappa:lambda ratio is normal, although clonality must be demonstrated within the cellular population identified by the disease-specific phenotype.

3 MBL lacking surface immunoglobulin is associated with CD5+CD23+ MBL.

4 A minimum of three colours (CD19 or CD20, anti-kappa and anti-lambda) should be used to confirm clonality, although four or more colours are preferable.

5 The fluorescence intensity of surface immunoglobulin, CD20 and CD79b expression if moderately increased should be noted.

6 The number of cells analysed should allow the formation of a cluster containing at least 50 events.

7 Repeat flow cytometric analysis is not necessary for research applications if monoclonality is confirmed by other approaches, e.g. fluorescence in situ hybridization or PCR, but may be useful for monitoring.

8 A disease-specific phenotype exists for hairy cell leukaemia (CD5+CD103+CD11c+CD25±) but it is probable that a full diagnosis of hairy cell leukaemia will be made in the presence of any level of circulating disease.

9 Other subclassifications may be included if sufficiently specific tests with evidence of a clinical association can be confirmed.

### Natural history of the disorder

As MBL has a greater prevalence than CLL, even if it were to be a precursor of CLL, a significant proportion of cases must either regress or remain static. This is in keeping with the finding that a large proportion of patients with early stage CLL show stable disease and, occasionally, decreasing CLL cell counts (Table I). Faguet et al (1992) reported a series of 39 patients with a slight increase in their absolute lymphocyte count; 24 had an abnormal B-cell clone and eight of the 24 patients progressed to clinical B-cell CLL over 5 years. In the limited NCI follow-up of familial MBL, progression to CLL has not yet been observed. Rawstron et al (2003) have begun to assess outcome in CD5+23+ MBL in a clinical population of patients who do not meet the diagnostic criteria for CLL. These are patients with an incidental finding of MBL meeting the criteria outlined above. Initial data (5-year median follow-up) suggests that approximately 60% have stable or regressive lymphocytosis; 35% show increasing lymphocyte cell counts, and 5% have required treatment. Of note, six of 47 patients
showed bi-phasic progression kinetics, with initially stable disease followed by a more pronounced increase in lymphocyte count at a median 3-5 years (range 0.5–7.0) from presentation, suggestive of a secondary event.

These data reinforce the concept that CLL presents with a very broad spectrum of tumour burden, and that risk of progression is independent of the absolute level at presentation. There is currently no evidence that early diagnosis of CLL is of any benefit to the patient. However, identifying CLL at its earliest stages is likely to be of significant value in identifying and defining the biological mechanisms responsible for disease aetiology and progression.

**Populations to consider**

One group (Kyle & Rajkumar, 1999; Kyle et al, 2002) established a significant relationship between multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS). Over a 30-year period, there is a 25-fold relative risk of MGUS progressing to MM and a 40-fold relative risk of developing macroglobulinaemia in subjects with IgM MGUS. At present, the notion that MBL is a precursor of CLL and other non-Hodgkin lymphomas (NHLs) is presumptive and a major goal of future studies should be to define this relationship. However, given the high population prevalence, screening of the general population would be prohibitively expensive and clinically uninformative and is not recommended outside of research applications. It is likely that, as with MGUS, MBL will predominantly be identified as an incidental finding in individuals being found medically for other reasons.

Epidemiological studies have shown that the risk of CLL in relatives of patients with CLL is increased and it is likely that a subset of the disease is caused by a genetic susceptibility (Fraumeni et al, 1969; Neuland et al, 1983; Blattner et al, 1987; Caporaso et al, 1991, 1997; Goldin et al, 1999; Yuille et al, 2000; Iihibe et al, 2001, 2002a,b). As CD5+23+ MBL may be a precursor of CLL, it has been postulated that MBL might be overrepresented in apparently healthy relatives of familial CLL cases. Such a proposition has recently been examined by two research groups. Rawstron et al (2002b) found that 14% of relatives of familial cases had MBL compared with 3% of outpatient controls. Marti et al (2003) also reported overrepresentation of MBL in relatives of familial cases (18% compared with 0.7% in controls). These studies provide strong support for the notion that MBL with a CLL-like phenotype may be a surrogate marker for carrier status. Therefore, there is a powerful rationale for research into the risk of progression for individuals with MBL in CLL families. However, it is still far from clear whether the detection of MBL predicts a higher probability of eventually developing CLL within CLL family members. Therefore, as with the general population, screening apparently healthy members from CLL families is currently not clinically informative.

It is recommended that relatives who are potential donors for allogeneic transplant be screened for MBL. This is primarily because the transplanted MBL is likely to impact on residual disease monitoring in the donor, although the potential for transplanted MBL to expand and or transform in an immunocompromised host is also of concern. Genetic counselling for a familial disorder could be considered for high-risk individuals in certain settings (Hampel et al, 2004).

**Recommendations for follow-up**

There are two key problems that arise in the area of asymptomatic or subclinical disorders: the first is what to inform the patient or individual, and the second is how closely the patient should be investigated and monitored.

The risk of progression to clinical disease is not known, and even if an association with clinical disease is convincingly demonstrated, it will be sometime before any risk of progression can be calculated. In the absence of precise clinical information about outcome, the adverse consequences of inducing anxiety by informing an individual that they might have a preclinical blood cancer or precursor condition; the lack of any effective early intervention; and the potential for adverse individual health insurance concerns, it may be unethical to inform patients of their disorder when it would result in little or no clinical benefit whilst incurring the risk of significant adverse effects. It is therefore acceptable in a research setting, where extremely low levels of monoclonal B cells may be identified, to not provide the results of investigations to patients. If MBL is diagnosed after specific investigation, it is important to stress that the risk of progression is not known but is likely to be very low and it may be helpful to offer periodic monitoring in these cases.

How closely patients are investigated and monitored is a significant problem. Although population screening is not clinically indicated, a substantial and increasing number of individuals are demonstrated to have MBL as an incidental finding during routine medical assessment. As this report is aimed at providing diagnostic criteria, it is not possible to provide evidence-based guidelines for monitoring patients with MBL. However, some suggestions can be provided based on initial studies.

The close relationship between CD5+23+ MBL and CLL suggests that the levels in the peripheral blood are likely to be indicative of bone marrow involvement. Therefore bone marrow investigation is unlikely to be of any value for these patients. B-lymphoproliferative disorders with a non-CLL phenotype may show much greater tissue or bone marrow involvement than the peripheral levels suggest, and therefore marrow investigation may be of value in patients with CD5+23+ or CD5- MBL. The presence of peripheral lymphadenopathy or organomegaly will have been excluded as part of the initial physical examination. However, in selected cases, a baseline chest X-ray, abdominal ultrasound and or a computed tomography scan may be indicated. And a rapid rise in the absolute lymphocyte count might warrant a diagnostic/prognostic bone marrow aspirate and biopsy.
For MBL patients identified during investigation of a mild lymphocytosis, it may be helpful to confirm whether the monoclonal B-cell counts are regressive, stable or progressive over a 3–6-month period. Long-term prospective studies will be required to determine whether periodic monitoring (i.e. as in MGUS, approximately yearly assessment of the absolute lymphocyte count and/or flow cytometry) will be of value in identifying the small proportion of MBL patients who will show progression to clinical disease.

Monoclonal B cells that are indistinguishable from early CLL/NHL may be detected in up to 4% of the general population. We propose the term MBL to describe this phenotype. MBL is detectable at a higher frequency in apparently unaffected members of CLL kindreds and is more prevalent with advancing age. Appropriate studies are needed to establish the natural history of MBL in both the general population and high-risk settings. The relationship of MBL to other non-CLL LPD, MGUS and reactive lymph node hyperplasia (Kussick et al, 2004) remains to be defined. Continued investigation of the human B-cell repertoire in a defined MBL population may reveal further abnormalities relevant to the aetiology and pathogenesis of CLL.

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